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REVIEW

Can metabolic plasticity be a cause for cancer? Warburg–Waddington legacy revisited

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Abstract Fermentation of glucose to lactate in the presence of sufficient oxygen, known as aerobic glycolysis or Warburg effect, is a universal phenotype of cancer cells. Understanding its origin and role in cellular immortalization and transformation has attracted considerable attention in the recent past. Intriguingly, while we now know that Warburg effect is essential for tumor growth and development, it is thought to arise because of genetic and/or epigenetic changes. In contrast to the above, we propose that Warburg effect can also arise due to normal biochemical fluctuations, independent of genetic and epigenetic changes. Cells that have acquired Warburg effect proliferate rapidly to give rise to a population of heterogeneous progenitors of cancer cells. Such cells also generate more lactate and alter the fitness landscape. This dynamic fitness landscape facilitates evolution of cancer cells from its progenitors, in a fashion analogous to Darwinian evolution. Thus, sporadic cancer can also occur first by the acquisition

of Warburg effect, then followed by mutation and selection. The idea proposed here circumvents the inherent difficulties associated with the current understanding of tumorigenesis, and is also consistent with many experimental and epidemiological observations. We discuss this model in the context of epigenetics as originally enunciated by Waddington.

Keywords Cancer epigenetics · Warburg effect · Crabtree effect · Cellular heterogeneity · Biological noise · Aerobic glycolysis · Bistability

Introduction

Current understanding of tumorigenesis is based on experimental, epidemiological, and evolutionary thoughts developed over the past few decades. Tumorigenesis appears to be due to random genetic and/or epigenetic alterations, and is thought to be a unidirectional process which occurs in a stepwise manner analogous to Darwinian evolution (Gatenby and Vincent 2003; Merlo et al. 2006). That is, normal cells acquire cancer phenotype because of mutations that bestow on them the fitness advantage to adapt to the constantly changing environmental and/or cellular constraints. Tumor cells are thought to originate from a common ancestor and are therefore functionally equivalent (Armitage and Doll 1954; Nowell 1976). The above model, commonly referred to as somatic mutation theory, was further supported with the identification of a large array of dominantly acting oncogenes and recessively acting tumor suppressor genes (Hahn and Weinberg 2002). As opposed to the “common ancestor” concept of somatic mutation theory, the cancer stem cell concept states that a subpopulation of cells of a tumor, referred to as cancer stem

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cells, derived from normal stem cells, or from differentiated cells due to random genetic/epigenetic events, gives rise to cancer cells incessantly (Reya et al. 2001; Bjerkvig et al. 2005; Feinberg et al. 2006; Dick 2008; Grønbaek et al. 2007). Although somatic mutation theory and cancer stem cell theory have been in vogue, many fundamental issues still remain unresolved (Soto and Sonnenschein 2004). Alternate possibilities that circumvent the inherent inconsistencies associated with the above models but do not strictly depend upon random genetic or epigenetic changes for initiating tumorigenesis has also been articulated (Kauffman 1971; Potter 2001; Laforge et al. 2005; Huang and Ingber 2007; Brock et al. 2009).

However, a close examination of the various models reveals that a unified concept of origin of cancer has not yet emerged, despite intense efforts to integrate a wealth of disparate data accumulated over the past several decades. Notwithstanding the presence of a bewildering array of genetic and epigenetic lesions, recent studies clearly point out that Warburg effect (conversion of glucose to lactate in the presence of oxygen), an essential feature of tumor cells, is thought to be a consequence of genetic or epigenetic alterations (Dang and Semenza 1999; Gatenby and Gillies 2004; Shaw 2006; Vander and Cantley 2009; Hsu and Sabatini 2008; Kaelin and Thompson 2010; Kroemer and Pouyssegur 2008). However, the intricate relationship that is observed between aerobic glycolysis and genetic alterations in cancer cells clearly points out that there exists a missing link between these two phenomena. Here, we propose that Warburg effect can also be a cause of cancer, and seek to unearth the “missing link” to explore its implications in the context of what we already know about cancer.

Warburg effect: fact or an artifact?

That Warburg effect occurs constitutively in cancer cells was first observed by Otto Warburg in the 1920s. This phenomenon was hypothesized to be due to an impairment in mitochondrial function (Warburg 1956). Because of the lack of experimental support, this idea was summarily abandoned (Weinhouse 1977). Initially, it was thought that the Warburg effect is an adaptation to hypoxic conditions, but later studies showed that mutations that lead to tumorigenesis also cause aerobic glycolysis by upregulating the expression of glycolytic genes at the transcriptional level (Semenza 2003). Warburg effect was initially thought to be incompatible with the concept of stepwise evolutionary progression of normal cells to cancer cells. This is because Warburg effect is an inefficient energy generating process, and also causes an acidic environment not suitable for cell proliferation by producing lactate. Therefore, it was

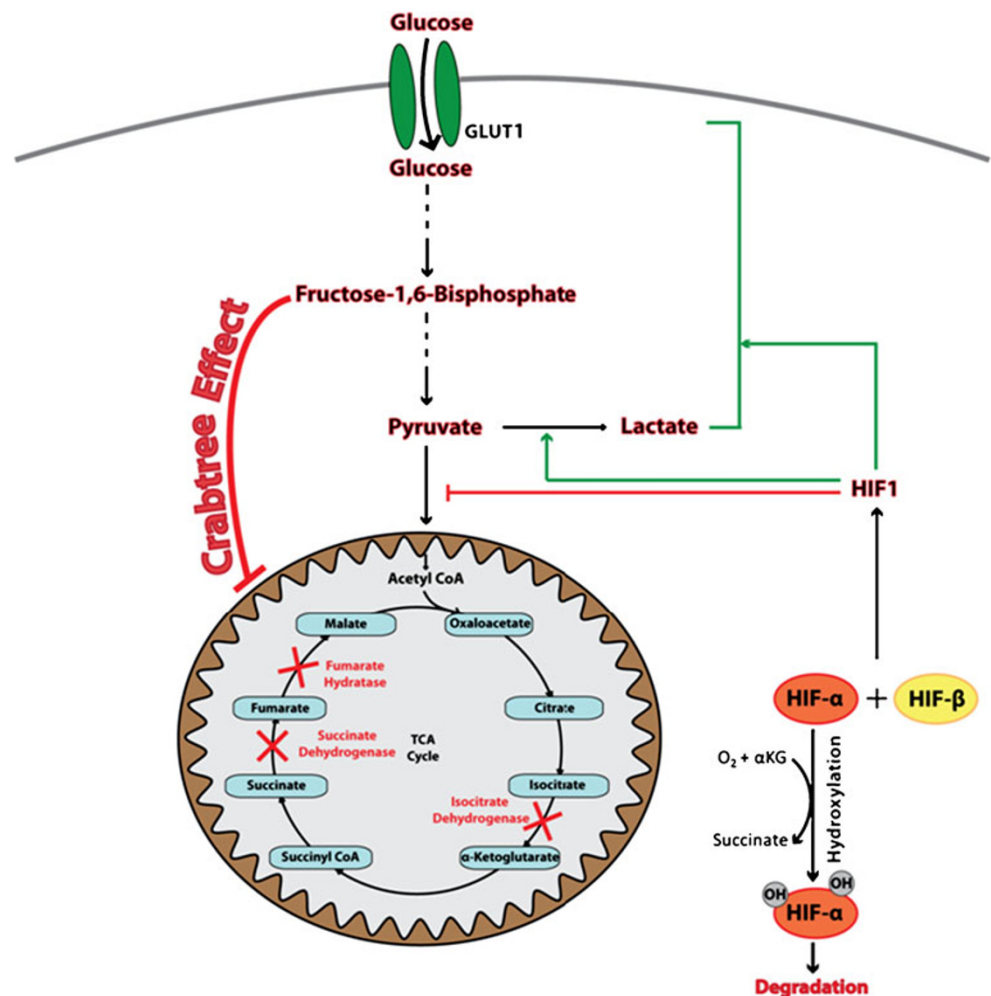
assumed that it cannot confer rapid proliferation and provide selective advantage to cancer cells. However, it is now widely accepted that, in fact, Warburg effect is one of the key features of tumorigenesis, and not only promotes rapid uncontrolled proliferation but also confers invasive property (Gatenby and Gillies 2004). In fact, solid tumors often exhibit areas of hypoxia and acidosis (Fang et al. 2008). Analysis based on evolutionary game theory and systems biology also points out the importance of Warburg effect in tumorigenesis (Basanta et al. 2008; Vazquez et al. 2010). Thus, we have come a long way in realizing that cancer cells cannot do away with aerobic glycolysis.

Molecular basis of the Warburg effect: metabolism versus mutation

Hypoxia-inducible factor (HIF1) was originally identified as a DNA-binding transcriptional factor which is present in nuclear extracts obtained from cells exposed to hypoxic conditions (Semenza and Wang 1992). It has been demonstrated that HIF1 activates the transcription of genes, required for the preferential diversion of glucose to lactate (see Fig. 1) and is a key factor in tumorigenesis (Kim et al. 2006; Semenza 2007; Baldewijns et al. 2010).

HIF1 is a heterodimer consisting of α and β subunits (Wang and Semenza 1995). While HIF1 β subunit is constitutively produced, the level of HIF1 α is determined by the rate of its degradation in oxygen (Wang et al. 1995). Its expression is known to increase exponentially in hypoxic conditions, but decays rapidly upon oxygenation with a half-life of 1–5 min, depending upon the experimental condition (Jiang et al. 1996). The degradation of HIF1 α is initiated by the hydroxylation of proline residues 402 and 564, which is required for the subsequent recognition by von Hippel–Landu tumor suppressor protein and proteasomal degradation (Jaakkola et al. 2001; Kaelin 2005). α -Ketoglutarate is one of the cosubstrates of the hydroxylation reactions, and succinate and CO₂ are the by-products (Bruick and McKnight 2001). Succinate and fumarate inhibit this hydroxylation step, and their increased levels, due to the mutations in the enzymes required for their synthesis, correlate with the incidence of cancer (King et al. 2006; Koivunen, et al. 2007). Recently, it has been observed that cells defective in either isocitrate dehydrogenase1 or 2 (ICD1, ICD2) also give rise to cancer probably because of lower levels of α ketoglutarate (Parsons et al. 2008; Zhao et al. 2009; Yan et al. 2009; Murugan et al. 2010). These discoveries point out that a normal level of α ketoglutarate plays a far more critical role in suppressing tumorigenesis, emphasizing that the link between metabolome and genome is far more complicated than what we originally believed. This

Fig. 1 Regulatory features of the altered fate of glucose metabolism in cancer cells. The lines with rightwards arrow indicate activation while lines with \perp indicate inhibition; α KG α ketoglutarate, *HIF1* hypoxia-inducible factor, *GLUT1* glucose transporter. *X* sign indicates the loss of function of the relevant enzymes



prompted us to look at the origin of cancer through the prism of Warburg effect; more specifically, the relationship between α ketoglutarate and HIF1.

Metabolic basis of the origin of cancer: an alternate possibility

It is clear from the foregoing section that the activation of HIF1 above a threshold would increase the flux of glucose towards lactate with a concomitant decrease towards oxidative phosphorylation (Fig. 1). Should this happen in normal cells due to noise, they would acquire aerobic glycolysis and proliferate rapidly, thus increasing the likelihood of incorporating genetic or epigenetic changes. During this process, the fitness landscape also is expected to change in a dynamic fashion. Under these altered cellular and environmental conditions, cells that have acquired fitness advantage due to genetic or epigenetic changes are likely to succeed. According to this view, the initial event in sporadic cancer is the fortuitous shift to aerobic glycolysis, which then provides a unique environment for cells to

proliferate rapidly and evolve in a cooperative fashion. It is to be noted that many studies have shown that cells with aerobic glycolysis have a clear proliferative advantage over cells that use mitochondrial oxidative phosphorylation (Pfeiffer et al. 2001; Frick and Schuster 2003; Molenaar et al. 2009).

In the light of Warburg effect, what needs to be understood is the teleological reason for recruiting of α -ketoglutarate as a cosubstrate to regulate the activity of HIF1. We suggest that by recruiting α -ketoglutarate as a cosubstrate, cell proliferation remains mechanistically coupled to energy production and metabolism. Whenever cells encounter low levels of α -ketoglutarate because of mitochondrial damage (for example, hypoxic conditions), an HIF1 pathway is automatically activated to increase aerobic glycolysis. It is pertinent to note here that metazoan mitochondria are prone to damage because of constant ROS generation, and accordingly, the mutation rate of mitochondrial genome is much higher as compared to the nuclear genome (Wolstenholme and Jeon 1992). In fact, HIF1 activity suppresses mitochondrial biogenesis and also aids in mitochondrial autophagy (Zhang et al. 2007, 2008).

Therefore, activating HIF1 in response to lowered α -ketoglutarate levels appears to be a part of the normal homeostatic mechanism. Such a mechanism allows the organism to proliferate rapidly by fermenting glucose to lactate, rather than to utilize glucose inefficiently, in case of a decrease in α ketoglutarate levels, due to intra- and/or extracellular cues.

Based on the above, we propose that activation of HIF1 ought to be sensitive to varying α -ketoglutarate levels, and we refer to this attribute as metabolic plasticity. This metabolic plasticity is a built-in feature that allows even normal cells to acquire aerobic glycolysis under many physiological conditions. For example, whenever cells need to proliferate rapidly, such as in wound healing or lymphocyte activation, cells switch over to aerobic glycolysis (Wang et al. 1976; Ghani et al. 2004). Recently, aerobic glycolysis has also been implicated in the establishment of CD8 T cell memory (Prlic and Bevan 2009). Thus, metabolic plasticity has evolved to allow cells to smoothly adapt to constantly varying intra- and extracellular conditions. That the HIF1 activity could be sensitive to the changes in α -ketoglutarate levels is reinforced by the observation that mutation in IDH1 or IDH2, but not both, are observed in gliomas (Kloosterhof et al. 2010). By implication, the remaining two ICDs are unable to maintain the concentration of α -ketoglutarate to a level that is sufficient to suppress HIF1 activity.

Because α -ketoglutarate is a key intermediate in energy metabolism as well as in biosynthesis, its concentration is expected to fluctuate either temporally or at the population level. If the concentration of α -ketoglutarate decreases to lower than a threshold which is normally observed, then HIF1 would be fortuitously activated, triggering Warburg effect, resulting in rapid cell proliferation. By the same token, it is also possible that this fortuitously acquired Warburg effect can wane as a function of time. However, in principle, Warburg effect, once initiated, can substitute for a mutation (similar to a recessive or a dominant mutation, as occurs in tumor suppressor genes or in oncogene activation, respectively), provided a self-sustaining mechanism stabilizes and propagates it during cell division. Thus, cells that have fortuitously acquired Warburg effect can serve as progenitors, and would evolve in Darwinian fashion to become cancer cells. According to this idea, a condition that temporarily impairs oxidative phosphorylation, (hypoxia, for example), can switch to aerobic glycolysis, eventually leading to cancer.

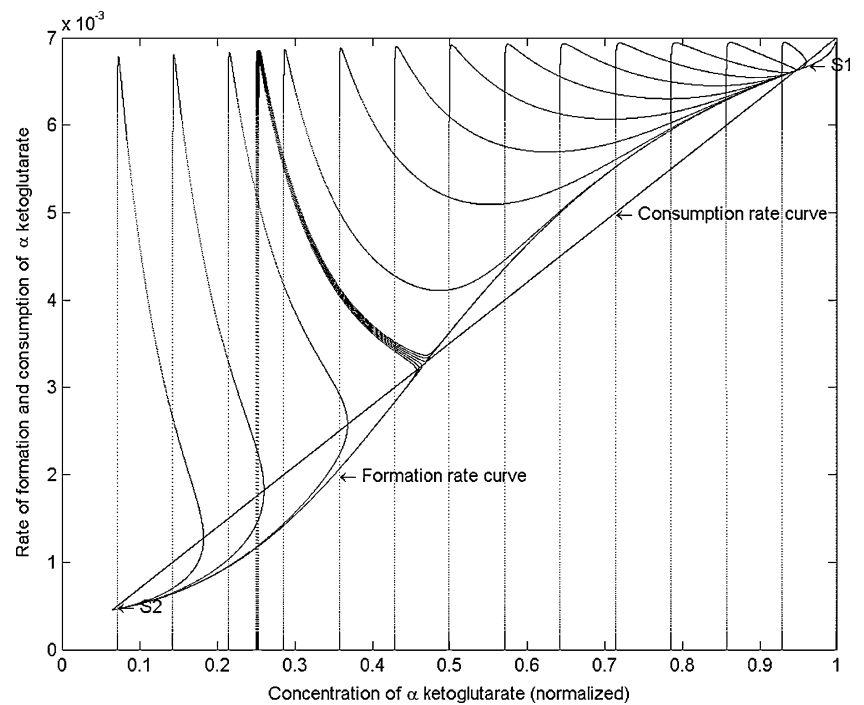
Persistence of aerobic glycolysis during cell division: epigenetic inheritance

A possible mechanism that can stabilize the fortuitously acquired aerobic glycolysis during cell division is through

feedback loops such as Crabtree effect (aerobic fermentation with high growth rate, Fig. 1). Crabtree effect is a well-known phenomenon in yeast (Pronk et al. 1996). It has been reported that under in vitro conditions, physiological levels of fructose1, 6-bisphosphate reduces the respiratory flux in isolated rat liver mitochondria (Díaz-Ruiz et al. 2008), indicating that Crabtree effect might even operate in humans. We have developed a theoretical model to demonstrate that the Crabtree effect, in principle, can sustain aerobic glycolysis. This model accounts for (1) glycolytic conversion of glucose to pyruvate and its subsequent conversion to lactate and α -ketoglutarate; (2) Hydroxylation of HIF 1 by α -ketoglutarate dependent proline hydroxylase and eventual degradation of HIF1; (3) the negative and positive feedback of HIF1 on oxidative phosphorylation and glucose uptake by the cell, respectively; and (4) the inhibition of TCA cycle performance through Crabtree effect due to increased flux through glycolysis. The analysis indicates that the system shows a bistable response, with two distinct stable states for various initial concentrations of α -ketoglutarate and HIF1 (Fig. 2). One of the stable states represents active oxidative phosphorylation with a concentration of α -ketoglutarate that is present in normal cells (Fig. 3a). As expected, HIF1 activity in these cells is low (Fig. S1, in supplementary information). The other stable state is characterized by cells with only 10% of the concentration of α -ketoglutarate, as observed in normal cells and with a 20-fold higher glucose uptake rate due to HIF1 activation (Fig. S2, in supplementary information). Under these conditions, the carbon flux is mainly routed towards lactate formation (Fig. 3b and details in Fig. S3, in supplementary information). It is clear from the analysis that the initial concentration of α -ketoglutarate dictates the attainment of either of the two states, with respect to HIF1 activity. Accordingly, when the concentration of α -ketoglutarate falls below a certain threshold (28% of the concentration observed in the normal cells, Fig. S1), the cells reside in the steady state characterized by higher glucose uptake rates, with the carbon flux shifting towards lactate formation.

The model was also utilized to determine the percentage of cells that would reside in the second phenotypic state by assuming a cell distribution with respect to the concentration of α -ketoglutarate (see Fig. S4, in supplementary information). It was quantified by the standard deviation around the mean, characterizing the concentration of α -ketoglutarate in a normal cell. The percentage of cells representing the phenotypic state of oxidative glycolysis increased exponentially with the standard deviation in the distribution, indicating a higher propensity for a shift from the normal behavior (Fig. S5, in the supplementary information). This analysis indicates that fluctuating levels of α -ketoglutarate can drive a cell to a new phenotype purely due to noise in the system.

Fig. 2 State space representation of initial levels of α ketoglutarate versus the rate of formation and consumption of α ketoglutarate. The rate of consumption of α ketoglutarate is invariant of the initial α ketoglutarate levels. On the other hand, the rate of formation of α ketoglutarate varies as a function of the initial levels of α ketoglutarate and eventually attains any one of the two stable states. An initial relative α ketoglutarate level above 0.28 would eventually attain state S1. On the other hand, an initial relative α ketoglutarate level below 0.28 would eventually attain the state S2. Thus, S1 and S2 represent the two stable states of α ketoglutarate levels indicating bistability



If a condition like hypoxia can shift the balance towards aerobic growth and eventually lead to cancer, one would expect the incidence of cancer cases to be far more than what is normally observed. However, it is important to keep in mind that the level of α -ketoglutarate would be much more tightly regulated than what we have demonstrated in the model. Nevertheless, our simple model, as it may seem,

has captured the intrinsic tendency of α -ketoglutarate to attain either of the two states. Here, we used Crabtree effect only to illustrate this point, because of its simplicity. But, other such feedback loops can also participate in sustaining the glycolytic phenotype. For example, AMP-dependent kinase, which responds to increased ATP level to suppress mitochondrial function, can also function in similar lines as

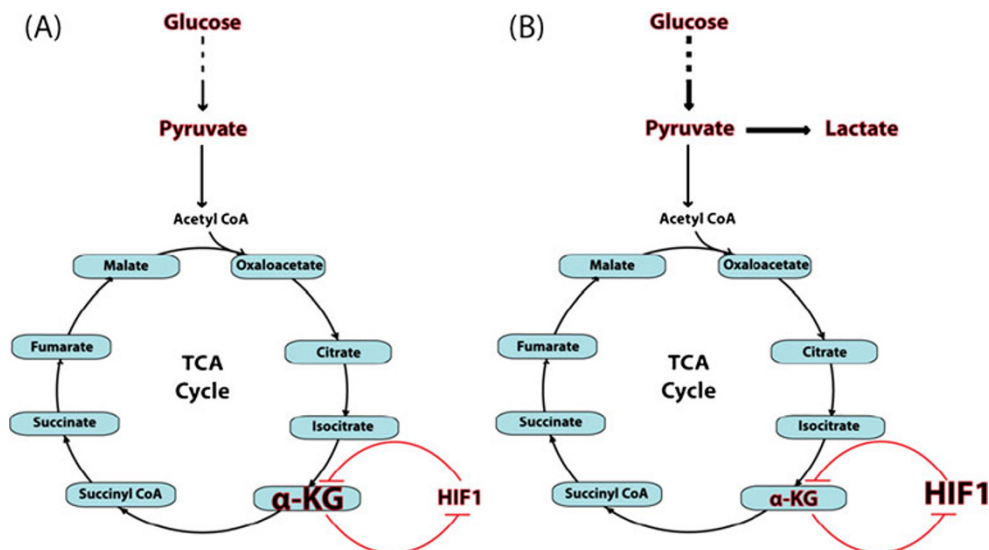


Fig. 3 Schematic representation of the regulatory circuit that gives rise to the bistability. α Ketoglutarate inhibits HIF1 by promoting its degradation. HIF1 inhibits the production of α ketoglutarate by inhibiting pyruvate entry into TCA. A decrease in α ketoglutarate below a threshold because of stochastic reasons would increase the HIF1 level, thus rendering a further decrease in α ketoglutarate (a).

This state corresponds to state S1 in Fig. 2. Conversely, α ketoglutarate concentration above a threshold will ensure that the HIF1-mediated negative feedback loop is abolished, thus increasing the levels of α ketoglutarate (b). This state corresponds to state S2 in Fig. 2. Double-negative feedback loops of the type shown above can force the system to exist in either of the two stable states

Crabtree effect (Hardie 2007). In fact, increase in HIF1 itself can act as a negative feedback loop, as mentioned earlier (Zhang et al. 2007). Similarly, it is also likely that loops that will force the system to attain the S1 state would also be operational. The approach discussed here now paves the way for a more detailed analysis of how HIF1 responds to different input signals at a systemic level.

We would like to emphasize that subsequent to the fortuitous metabolic shift, additional mechanisms can also come into play to stabilize the aerobic glycolysis. For example, in breast cancer cells, the downregulation of two isozymes of fructose 1,6 bisphosphatase (FBPase), due to the methylation of CpG islands of the promoters of both of the isozymes of FBPase, has been documented (Bigl et al. 2008). This could further lead to the accumulation of fructose 1,6 biphosphate, thus exacerbating the Crabtree effect discussed earlier. In another example, Hexokinase 2, one of the key enzymes thought to be responsible for Warburg effect (see below for details), has also been shown to be upregulated through epigenetic mechanisms (Goel et al. 2003). Similarly, alteration in the expression of a large array of genes through epigenetic modification has been reported to be responsible for initiation and progression of tumorigenesis. Thus, a fortuitous metabolic shift followed by epigenetic modification and genetic alterations seems to be the sequence of events that occurs in cases of sporadic cancers.

Need for an alternate model: new perspectives

According to our model, aerobic glycolysis precedes genetic or epigenetic changes, and this has important implications with respect to apoptosis. Independent studies have reported that high glycolytic rate is anti-apoptotic, suggesting that cells destined to become cancerous a priori escape from apoptosis. For example, the role of hexokinase II (HXKII) and glucose metabolism in aerobic glycolysis is highlighted by the observation that H-19 hepatoma cells produce more lactate when glucose is used as the carbon source as compared to galactose. Consistent with this, HXII protein, an apoptotic suppressor, is associated with mitochondrial fraction only when glucose but not galactose was used as the carbon source, indicating that apoptotic suppression is specific to glucose metabolism (Bustamante and Pedersen 1977; Chiara et al. 2008). In an independent experiment, over-expression of GLUT1/HEX1 led to significantly increased lactate with near normal or reduced TCA metabolites, with concomitant suppression of apoptosis (Zhao et al. 2007). Glucose analog did not achieve the same effect, indicating that suppression of apoptosis is specific to glucose metabolism. It has been demonstrated that increased glucose metabolism, observed in neuronal

and cancer cells, suppresses apoptosis by keeping the cytochrome C, a proapoptotic factor, in its reduced state (Vaughn and Deshmukh 2008). Further, glucose has also been shown to suppress the apoptosis induced by p53-dependent PUMA (Zhao et al. 2008). Therefore, we speculate that increased aerobic glycolysis occurring a priori can prevent the cells from entering into apoptosis, thus obviating the need to invoke alternate mechanisms for the suppression of apoptosis observed in cancer.

Our model suggests that the tumor population is per se heterogeneous, as there is no reason to believe that all progenitors having aerobic glycolysis ought to give rise to cancer. Cancer stem cells are notoriously heterogeneous in terms of tumorigenicity in xenograft experiments, as well as in their ability to express tumor antigens (Dick 2008; Rosen and Jordan 2009). While the molecular basis of this heterogeneity is not clear, it is thought to arise because of genetic and epigenetic changes. The heterogeneity has also been explained based on network dynamics or multiplicity of stable genetic networks (Huang and Ingber 2007). According to our model, the heterogeneity could as well reflect cells trapped in different metabolic steady states and later stabilized by stable genetic networks or genetic or epigenetic processes. It is now becoming increasingly clear that heterogeneity at the metabolic level is a desirable property for tumors. For example, while some cells of tumor population produce lactate, others consume it. Interestingly, interfering with this metabolic symbiosis by blocking lactate uptake suppresses tumor growth, indicating that such a metabolic heterogeneity has a distinct advantage to the tumor as a whole (Semenza 2008). Recently, a similar metabolic coupling has been observed in breast cancer (Pavlidis et al. 2009), where it has been suggested that the metabolic dependency is well-suited for the overall growth of tumor cells. As mentioned earlier, models based on game theory have proposed that the invasive phenotype is more likely to succeed in a tumor population consisting of cells with autonomous growth, glycolytic phenotype, and invasive phenotype. Intriguingly, it has been demonstrated that expression of frataxin, an upregulator of oxidative phosphorylation in colon cancer lines, showed reduced capacity for tumor formation (Schulz et al. 2008). An explanation for the above findings is that the over-expression of frataxin could specifically activate oxidative phosphorylation in cells that acquired aerobic glycolysis, thus interfering with the metabolic coupling.

Existence of heterogeneity in tumor population can also explain the phenomenon of reversion of cancer cells to normal cells (Kenny and Bissell 2003). For example, those cells existing in aerobic glycolytic state that have not made a genetic commitment to be cancerous can revert back to the normal phenotype. It has been demonstrated that mouse embryonic carcinoma cells, which otherwise form tumors

upon subcutaneous injection, could develop if injected into blastocysts (Brinster 1974). The subsequent study which confirmed this observation suggested a non-mutational basis for transformation to malignancy and its reversal to normalcy (Mintz and Illmensee 1975).

Based on our model, we provide an alternate possibility for the two-hit hypothesis of Knudson (Knudson 1971), with respect to p53 haploinsufficiency. Theoretical analysis shows that in general, haploinsufficiency increases transcriptional noise (Cook et al. 1998). One of the roles of p53 is to increase mitochondrial oxygen uptake by activating the transcription of SCO2, an assembly protein required for the synthesis of cytochrome c oxidase 2 (Matoba et al. 2006). Based on this, we speculate that the p53 haploinsufficient cell population would show a wider cell distribution with respect to aerobic glycolysis, since SCO2 is a component of oxygen uptake apparatus. Accordingly, the p53 haploinsufficient cell population is expected to have a higher fraction of cells existing in aerobic glycolysis because of low oxygen uptake. These cells would proliferate rapidly, thus increasing the probability of a mutation in the existing TP53 copy. That is, p53 haploinsufficient cells favor aerobic glycolysis because of inherent noise. This idea is compatible with the finding that haploinsufficiency of NF1 has been shown to increase the rate of formation of tumors because of increased noise (Kemkemer et al. 2002). A prediction from our hypothesis is that metformin, a drug known to promote mitochondrial oxidation by activating AMP-dependent kinase, which is an upstream activator of p53 (Jones et al. 2005), should reduce the incidence of tumors in individuals known to have only one allele of TP53.

A direct prediction of our model is that an increase in the oxidative phosphorylation would decrease or suppress the incidence of cancer, as has been reported in literature (Schulz et al. 2008). In contrast, an increase in the glycolytic flux would increase the incidence of cancer. Many independent circumstantial and experimental observations support this correlation. For example, more than 90% of ovarian cancer is due to ovulation-induced wound repair (Godwin et al. 1993). This could be because of increased aerobic glycolysis associated with cell proliferation which occurs during wound repair, thereby increasing the incidence of cancer. Immortalization, the first step in tumorigenesis, so far thought to occur because of mutations (Carnero and Lleónart 2010), has been demonstrated to occur upon overexpression of phosphoglycerate mutase or phosphoglucosomerase (Kondoh et al. 2005). As expected from our model, this overexpression increased the glycolytic flux with concomitant reduction in oxidative metabolism. Epidemiological data indicate that the incidence of cancer-related mortality was higher in patients with type 2 diabetes who use insulin or sulfonylureas (Bowker et al.

2006). This could be because of increased uptake of glucose, which exerts Crabtree effect, leading to glycolytic phenotype. Conversely, diabetic patients consuming metformin show statistically significant reduction in tumor incidence (Evans et al. 2005). This effect of metformin could be a secondary consequence of increased mitochondrial oxidation of glucose, thereby preventing cells from entering into glycolytic phenotype. In light of the above, it is imperative that we take a closer look at the role of metabolism in the pathophysiology of cancer.

We suggest that cells which have fortuitously acquired aerobic glycolysis proliferate rapidly, alter the fitness landscape and serve as precursors of cancer cells. Our model is not only compatible with the available data, but also satisfies three essential conditions required for the origin and evolution of cancer cells. First, acquisition of glycolytic phenotype would alter the fitness landscape by producing lactate. Second, the cells that have acquired aerobic glycolysis divide rapidly, thereby increasing the genetic and/or epigenetic variants, which then get selected in a dynamic fitness landscape. Third, metabolic heterogeneity of tumor cell population is an intrinsic feature of our model, and appears to play a significant role in the pathophysiology of cancer. Thus, the basic tenet of our model that the genetic and/or epigenetic changes can occur subsequent to the cells acquiring aerobic glycolysis unifies our current understanding of the origin and progression of cancer. Further, it places the concept that the “origin and progression of cancer is analogous to Darwinian evolution” on firmer ground, with the exception that the initial event can also be nongenetic.

Conclusions

While the importance of Warburg effect in tumor metabolism is now widely accepted, it is still tacitly believed that it occurs subsequent to genetic or epigenetic changes during tumorigenesis. On the other hand, in spite of intense efforts and a wealth of data, we continue to grapple with the idea that genetic and epigenetic changes are the root causes of cancer. This dichotomy has only widened the gap between the genetic and metabolic basis of cancer. Our hypothesis that because of noise, a stable glycolytic phenotype can be manifested and propagated during cell division in normal cells, independent of genetic or epigenetic event, provides a conceptually new avenue that would radically alter the way we think about cancer. We point out that metabolomes and genomes are intricately intertwined at the functional level, and attempts to subjugate metabolism as only a secondary consequence of genetic or epigenetic changes is unlikely to bear fruits. It appears that the major target of evolutionary force during evolution of eucaryotes are the regulatory

circuits that link metabolism, which provides the cellular fabric plus fuel with that of the genome, which encapsulates the genetic program. That enzymes involved in glycolysis and TCA are moonlighting proteins (Sriram et al. 2005; Gancedo and Flores 2008) with diverse roles, such as transcriptional activation, suppression of apoptosis etc., supports the above conjecture. In light of this, it is imperative to consider metabolism as a prime player in the overall orchestration of many aberrant biological processes, including tumorigenesis. Therefore, we suggest that Warburg effect be considered more as a causative agent than as a metabolic shift required only for tumor progression.

Attempts to elucidate the role of cytoplasmic factors in dictating the phenotypic outcome invariably takes us back to the fundamental observations made by Waddington in 1950s. He coined the term “epigenotype”, specifically to highlight the role of cytoplasmic factors in the eventual translation of the genotype to the phenotype. The molecular basis of this concept is only now beginning to be understood in greater detail. The above concept was developed in an attempt to describe the various developmental fates originating from a given genotype. To explain this, he used the metaphor “epigenetic landscape” to refer to the possible phenotypic states that a cell can potentially attain (reviewed in Gilbert 2000). One may consider a cancerous state as one of the aberrant developmental options available to a normal cell (Kauffman 1971). However, because of canalization, or the robustness of developmental pathways, cells normally do not take this aberrant route; that is, invariably the cells are canalized to follow the destined developmental trajectory under normal conditions, but if the cellular conditions are altered beyond a threshold, they may attain an aberrant phenotypic state. Thus, based on the fundamental ideas of Warburg and Waddington, as well as our current understanding, we have synthesized a novel concept which has far-reaching ramifications, as far as cancer biology is concerned.

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Conflict of interest The authors declare that they have no conflict of interest.

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